

Interleukin-5 Is Essential for Vaccine-Mediated Immunity but Not Innate Resistance to a Filarial Parasite

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The study of protective immune mechanisms effective against filarial nematodes has been hampered by the inability of these important human pathogens to infect laboratory mice. Recently, *Litomosoides sigmodontis*, a natural parasite of rats, has been developed as a valuable model for the study of filarial infection. BALB/c mice are fully susceptible to infection with *L. sigmodontis* third-stage larvae and develop patent infection. In contrast, mice on the C57BL background are resistant, and parasites undergo only a single molt and do not mature to adulthood. We used interleukin-5 (IL-5)-deficient mice on the C57BL/6 background to address the role of IL-5 and eosinophils in the innate resistance of C57BL/6 mice. We found no differences in parasite survival between IL-5-deficient and C57BL/6 mice. However, when these mice were used for the analysis of vaccine-mediated immunity, a critical role for IL-5 was elucidated. Mice genetically deficient in IL-5 were unable to generate a protective immune response when vaccinated with irradiated larvae, whereas C57BL/6 mice were fully protected from challenge infection. These studies help to clarify the highly controversial role of eosinophils in filarial infection.

Filarial nematodes are the causative agents of lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness). Together, these parasites (*Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*) afflict more than 140 million people worldwide (37). A major stumbling block in the study of filarial disease is the inability of these human pathogens to establish infection in well-characterized laboratory animals. A recent advance in filariasis research has been the development of a murine model of infection, using the rodent filarial parasite *Litomosoides sigmodontis* (24). *L. sigmodontis* is the only filarial species able to complete its full development cycle in inbred laboratory mice.

One of the benefits of this new model is that susceptibility to infection is murine strain dependent, allowing genetic dissection of the mechanisms that determine innate resistance as has been done for other parasitic systems such as *Trichuris muris* and *Leishmania major* (11, 30). BALB/c mice are fully susceptible to *L. sigmodontis* infection, and parasites develop through to patency. In contrast, mice on the C57BL background are resistant and patent infections are never seen (27). Interestingly, this pattern of resistance and susceptibility is similar to that seen for the protozoan parasite *L. major* and opposite to that of the intestinal nematode *T. muris*.

How cells of the innate immune system (e.g., granulocytes and macrophages) might mediate protective immunity to nematode parasites is an unresolved and controversial issue. In particular, despite their distinctive association with nematode infection, the exact role of eosinophils is not established for either intestinal or tissue locales and remains an area of considerable scientific debate (3, 16, 35). Numerous studies have shown that the characteristic eosinophilia observed in nema-

tode infection is dependent on host interleukin-5 (IL-5) (7, 15, 16). Not surprisingly, eosinophil recruitment to filarial infection is also IL-5 dependent (12, 22). However, without the ability to use the extensive array of murine reagents, the exact function of eosinophils in immunity to infection has been difficult to establish.

We have chosen the *L. sigmodontis* model to directly address the role of eosinophils in resistance to infection with vector-derived larvae. We hypothesized that if eosinophils were a key player in innate resistance to filarial parasites, then a genetic inability to recruit eosinophils might render resistant C57BL/6 mice more susceptible to infection. In this study, we demonstrate that parasite survival following primary infection of IL-5-deficient mice on the C57BL/6 background does not differ from that in infections in wild-type (WT) C57BL/6 mice. Parasite attrition in naive resistant mice normally occurs after larval migration and molting (23). In immunized mice, however, parasites die rapidly at the site of inoculation with eosinophils surrounding the dying larvae (20). We therefore chose to use these mice to additionally investigate vaccine-mediated protection. We found that in contrast to innate resistance, the rapid killing of larvae in vaccinated mice was highly dependent on IL-5.

MATERIALS AND METHODS

Parasites and mouse infection model. *L. sigmodontis* is transmitted by the mite vector *Omithonyssus bacoti*. Infective third-stage larvae (L3) migrate through the lymphatics to the thoracic cavity, where larvae mature to the adult stage. Adult parasites produce microfilariae that circulate in the bloodstream. Laboratory maintenance of *L. sigmodontis* and recovery of infective larvae from the mite vector were carried out as previously described (9, 27). IL-5-deficient mice on the C57BL/6 background were the kind gift of Manfred Kopf (Basel, Switzerland). All mice (C57BL/6 and IL-5 deficient) were bred on site, and 6- to 8-week-old males were used for all experiments.

(i) Primary infection. In each experiment, age-matched groups of IL-5-deficient and WT C57BL/6 mice were infected subcutaneously with 25 infective larvae. Necropsies were performed 10, 20, or 40 days postinfection. For all experiments, the number of worms that had developed from infective larvae was counted as described by Bain et al. (4). Briefly, all dissections were performed in

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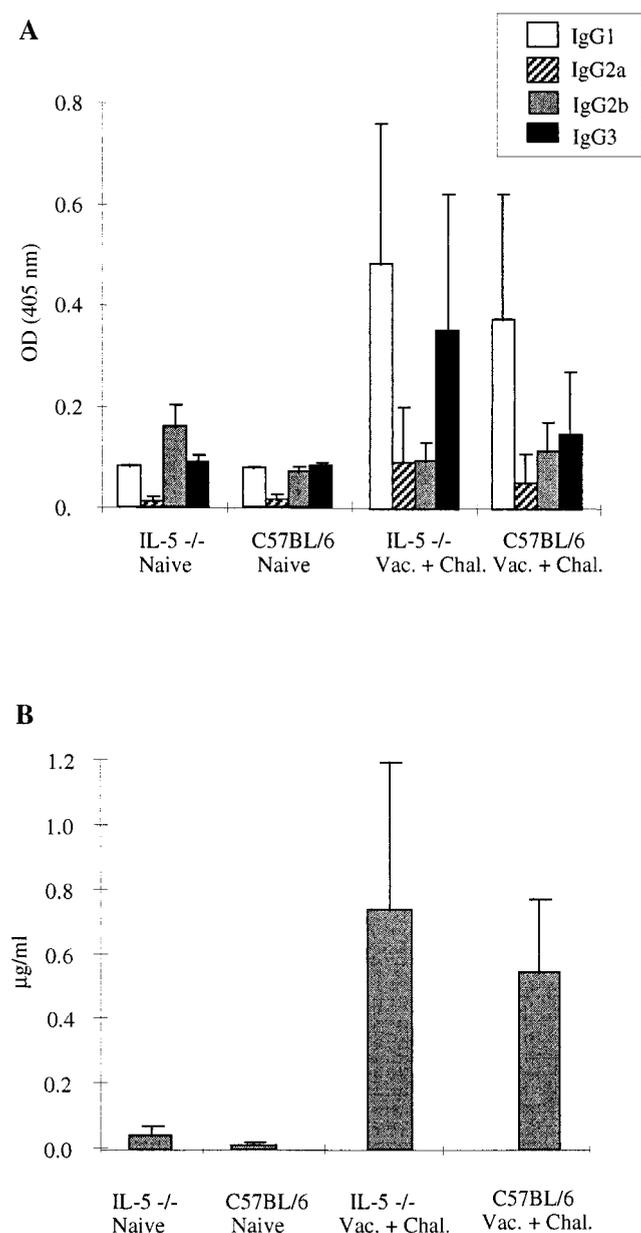


FIG. 4. Antibody isotype production in vaccinated IL-5-deficient and WT mice 10 days following challenge (chal.) infection. (A) *L. sigmondontis*-specific IgG subclass responses; (B) total serum IgE. Values are expressed as the mean \pm standard deviation of five mice per group. IgG1, IgG3, and IgE were significantly higher in vaccinated (Vac.) mice than naive mice ($P < 0.05$). OD, optical density.

failure of vaccine-mediated protection is due to insufficient numbers of eosinophils. Additionally, insufficient antibody responses in the IL-4-deficient mice may fail to trigger eosinophils.

Previous work in the *L. sigmondontis* vaccination model has shown that eosinophils are present and degranulate at the site of challenge infection (20). This observation in combination with our data here using mice genetically deficient in IL-5 and the work of others (14, 17) provides strong evidence that eosinophils are responsible for vaccine-mediated protection in filarial infection of mice. Killing of incoming larvae by eosinophils may be mediated via antibodies, as previous studies have shown reduced protection when heterologous larvae are used

in the challenge (33) and eosinophils alone could not account for this specific immune recognition. However, we saw no significant differences in antibody levels or isotype distribution between WT and IL-5-deficient mice. These data suggest that the failure to achieve protection in the gene-deficient mice is likely due to the absence of eosinophils rather than an IL-5-mediated defect in B-cell function.

Nonetheless, it will be necessary to rule out other effects of IL-5 such as defects due to B-1 cells (15). In addition, IL-5-deficient mice infected with *Toxoplasma gondii* failed to make antigen-specific IgG1 in contrast to WT controls (28), consistent with previous in vitro studies (39). However, IgG1 levels were unimpaired in IL-5-deficient mice during *L. sigmondontis* infection, perhaps because very high levels of IL-4 are induced by nematode infection. IL-5 may be important only if levels of IL-4 are suboptimal. Although our data show no evidence for differences in antibody responsiveness between IL-5-deficient and WT mice, we cannot exclude the possibility that qualitative effects of IL-5 on antibody profiles are responsible for the protective immune response.

It is important to consider why eosinophils appear to contribute to the early response in vaccinated mice but are not important in the late primary response to the parasite. In both situations, parasite-specific antibody and eosinophils are present. Several factors may be responsible for this difference. First, the immune responses to important target antigens may be considerably greater in repeatedly vaccinated mice than in a late primary infection. Second, infective larvae may be intrinsically more susceptible to eosinophil-mediated killing than the later stages of development. Third, the location of the more mature parasites (outside the subcutaneous tissue) may facilitate their ability to mechanically avoid eosinophil attack. In addition, later in the infection process, immune suppressive mechanisms may counter important host effector mechanisms. Finally, the ability of IL-5-deficient mice to clear a primary infection does not rule out a role for eosinophils in a normal setting, as other mediators may be readily able to compensate for a lack of eosinophils in the gene-deficient mice.

The data on the role of IL-5 using both neutralizing antibodies and transgenic mice have not generated a consistent picture between different nematode species and even within parasite families (8). For example, our data contrast directly with those for *Strongyloides ratti* infection in mice, where IL-5 is important in the primary infection but is not a player in the protective response against a challenge infection (26). This is not surprising, as each parasite has evolved independently and has developed disparate survival strategies that may or may not involve direct avoidance of eosinophil-mediated killing. Further, the location and migration patterns of the parasite life cycle stages are certain to influence their susceptibility to a particular innate or adaptive immune mechanism. It is becoming apparent, therefore, that conclusions found within one system cannot be readily applied to another. However, we do hope that the data provided in this report will help to sharpen our image of the mechanisms involved in the destruction of filarial nematodes and help the development of effective strategies to prevent new infections.

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REFERENCES

- Abraham, D., M. L. Eberhard, A. M. Lange, W. Yutanawiboonchai, F. B. Perler, and J. B. Lok. 1992. Identification of surrogate rodent hosts for larval *Onchocerca lienalis* and induction of protective immunity in a model system. *J. Parasitol.* **78**:447–453.
- Al-Qaoud, K. M., A. Taubert, H. Zahner, B. Fleischer, and A. Hoerauf. 1997. Infection of BALB/c mice with the filarial nematode *Litomosoides sigmodontis*: role of CD4⁺ T cells in controlling larval development. *Infect. Immun.* **65**:2457–2461.
- Allen, J. E., and R. M. Maizels. 1997. Th1-Th2: reliable paradigm or dangerous dogma? *Immunol. Today* **18**:387–392.
- Bain, O., S. Wanji, P. N. Vuong, P. Maréchal, L. Le Goff, and G. Petit. 1994. Larval biology of six filariae of the sub-family Onchocercinae in a vertebrate host. *Parasite* **1**:241–254.
- Bancroft, A. J., R. K. Grencis, K. J. Else, and E. Devaney. 1993. Cytokine production in BALB/c mice immunized with radiation attenuated third stage larvae of the filarial nematode, *Brugia pahangi*. *J. Immunol.* **150**:1395–1402.
- Behnke, J. M., C. J. Barnard, and D. Wakelin. 1992. Understanding chronic nematode infections—evolutionary considerations, current hypotheses and the way forward. *Int. J. Parasitol.* **22**:861–907.
- Coffman, R. L., B. W. P. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* **245**:308–310.
- Dent, L. A., C. M. Daly, G. Mayrhofer, T. Zimmerman, A. Hallett, L. P. Bignold, J. Creaney, and J. C. Parsons. 1999. Interleukin-5 transgenic mice show enhanced resistance to primary infections with *Nippostrongylus brasiliensis* but not primary infections with *Toxocara canis*. *Infect. Immun.* **67**:989–993.
- Diagne, M., G. Petit, P. Liot, J. Cabaret, and O. Bain. 1990. The filaria *Litomosoides galizai* in mites; microfilarial distribution in the host and regulation of the transmission. *Ann. Parasitol. Hum. Comp.* **65**:193–199.
- Folkard, S. G., P. J. Hogarth, M. J. Taylor, and A. E. Bianco. 1996. Eosinophils are the major effector cells of immunity to microfilariae in a mouse model of onchocerciasis. *Parasitology* **112**:323–329.
- Grencis, R. K. 1996. T cell and cytokine basis of host variability in response to intestinal nematode infections. *Parasitology* **112**:S31–S37.
- Hall, L. R., R. K. Mehlotra, A. W. Higgins, M. A. Haxhiu, and E. Pearlman. 1998. An essential role for interleukin-5 and eosinophils in helminth-induced airway hyperresponsiveness. *Infect. Immun.* **66**:4425–4430.
- Hogarth, P. J., M. J. Taylor, and A. E. Bianco. 1998. IL-5-dependent immunity to microfilariae is independent of IL-4 in a mouse model of onchocerciasis. *J. Immunol.* **160**:5436–5440.
- Johnson, E. H., S. Schynder-Candrian, T. V. Rajan, F. K. Nelson, S. Lustigman, and D. Abraham. 1998. Immune responses to third stage larvae of *Onchocerca volvulus* in interferon- γ and interleukin-4 knockout mice. *Parasite Immunol.* **20**:319–324.
- Kopf, M., F. Brombacher, P. D. Hodgkin, A. J. Ramsay, E. A. Milbourne, W. J. Dai, K. S. Ovington, C. A. Behm, G. Kohler, I. G. Young, and K. I. Matthaeci. 1996. IL-5-deficient mice have a developmental defect in CD5⁺ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* **4**:15–24.
- Korenaga, M., and I. Tada. 1994. The role of IL-5 in the immune responses to nematodes in rodents. *Parasitol. Today* **10**:234–236.
- Lange, A. M., W. Yutanawiboonchai, P. Scott, and D. Abraham. 1994. IL-4- and IL-5-dependent protective immunity to *Onchocerca volvulus* infective larvae in BALB/cBYJ mice. *J. Immunol.* **153**:205–211.
- Lawrence, R. A., J. E. Allen, J. Osborne, and R. M. Maizels. 1994. Adult and microfilarial stages of the filarial parasite *Brugia malayi* stimulate contrasting cytokine and Ig isotype responses in BALB/c mice. *J. Immunol.* **153**:1216–1224.
- Le Goff, L., P. Maréchal, G. Petit, D. W. Taylor, W. Hoffman, and O. Bain. 1997. Early reduction of the challenge recovery rate following immunization with irradiated infective larvae in a filaria mouse system. *Trop. Med. Int. Health* **2**:1170–1174.
- Le Goff, L., C. Martin, I. P. Oswald, P. N. Vuong, G. Petit, M. N. Ungeheuer, and O. Bain. Parasitology and immunology of mice vaccinated with irradiated *Litomosoides sigmodontis* larvae. *Parasitology*, in press.
- Lucius, R., G. Textor, A. Kern, and C. Kirsten. 1991. *Acanthocheilonema viteae*: vaccination of jirds with irradiation-attenuated stage-3 larvae and with exported larval antigens. *Exp. Parasitol.* **73**:184–196.
- MacDonald, A. S., P. Loke, and J. E. Allen. Suppressive antigen-presenting cells in helminth infection. *Pathobiology*, in press.
- Maréchal, P., L. Le Goff, G. Petit, M. Diagne, D. W. Taylor, and O. Bain. 1996. The fate of the filaria *Litomosoides sigmodontis* in susceptible and naturally resistant mice. *Parasite* **3**:25–31.
- Maréchal, P., G. Petit, M. Diagne, D. W. Taylor, and O. Bain. 1994. Use of the *Litomosoides sigmodontis*-mouse model in development of an *Onchocerca* vaccine. II. *L. sigmodontis* in the BALB/c mouse: vaccination experiments; preliminary immunological studies. *Parasite* **1**:31–32.
- McLeod, R., E. Buschman, L. D. Arbuckle, and E. Skamene. 1995. Immunogenetics in the analysis of resistance to intracellular pathogens. *Curr. Opin. Immunol.* **7**:539–552.
- Ovington, K. S., K. McKie, K. I. Matthaeci, I. G. Young, and C. A. Behm. 1998. Regulation of primary *Strongyloides ratti* infections in mice: a role for interleukin-5. *Immunology* **95**:488–493.
- Petit, G., M. Diagne, P. Maréchal, D. Owen, D. Taylor, and O. Bain. 1992. Maturation of the filaria *Litomosoides sigmodontis* in BALB/c mice; comparative susceptibility of nine other inbred strains. *Ann. Parasitol. Hum. Comp.* **67**:144–150.
- Purkerson, J. M., and P. C. Isakson. 1992. Interleukin 5 (IL-5) provides a signal that is required in addition to IL-4 for isotype switching to immunoglobulin (Ig) G1 and IgE. *J. Exp. Med.* **175**:973–982.
- Rajan, T. V., P. Porte, J. A. Yates, L. Keefer, and L. D. Shultz. 1996. Role of nitric oxide in host defense against an extracellular, metazoan parasite, *Brugia malayi*. *Infect. Immun.* **64**:3351–3353.
- Reiner, S. L., and R. M. Locksley. 1995. The regulation of immunity to *Leishmania major*. *Annu. Rev. Immunol.* **13**:151–177.
- Sartono, E., Y. C. M. Kruize, A. Kurniawan-Atmadja, R. M. Maizels, and M. Yazdanbakhsh. 1996. Depression of antigen-specific interleukin-5 and interferon- γ responses in human lymphatic filariasis as a function of clinical status and age. *J. Infect. Dis.* **175**:1276–1280.
- Schrempf-Eppstein, B., A. Kern, G. Textor, and R. Lucius. 1997. *Acanthocheilonema viteae*: vaccination with irradiated L3 induces resistance in three species of rodents (*Meriones unguiculatus*, *Mastomys coucha*, *Mesocricetus auratus*). *Trop. Med. Int. Health* **2**:104–110.
- Storey, D. M., and A. S. Al-Mukhtar. 1982. Vaccination of jirds, *Meriones unguiculatus*, against *Litomosoides carinii* and *Brugia pahangi* using irradiated larvae of *L. carinii*. *Tropenmed. Parasitol.* **33**:23–24.
- Taylor, M. J., R. P. van Es, K. Shay, S. G. Folkard, S. Townson, and A. E. Bianco. 1994. Protective immunity against *Onchocerca volvulus* and *O. lienalis* infective larvae in mice. *Trop. Med. Parasitol.* **45**:17–23.
- Urban, J. F., K. B. Madden, A. Sveti'c, A. Cheever, P. P. Trotta, W. C. Gause, I. M. Katona, and F. D. Finkelman. 1992. The importance of Th2 cytokines in protective immunity to nematodes. *Immunol. Rev.* **127**:205–220.
- Weil, G. J., B. W. Li, F. Liftis, and R. Chandrashekar. 1992. *Brugia malayi*—antibody responses to larval antigens in infected and immunized jirds. *Exp. Parasitol.* **74**:315–323.
- World Health Organization. 1992. Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. WHO Tech. Rep. Ser. **821**:1–71.
- Yates, J. A., and G. I. Higashi. 1985. *Brugia malayi*: vaccination of jirds with ⁶⁰Co-attenuated infective stage larvae protects against homologous challenge. *Am. J. Trop. Med. Hyg.* **34**:1132–1137.
- Zhang, Y., and E. Y. Denkers. 1999. Protective role for interleukin-5 during chronic *Toxoplasma gondii* infection. *Infect. Immun.* **67**:4383–4392.